Could medical intervention work for aortic aneurysms?

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Manuscript received July 20, 2004; revised manuscript August 7, 2004

Presented at the 56th Annual Meeting of the Southwestern Surgical Congress, Monterey, California, April 18–21, 2004

Abstract

Background: Aortic aneurysms represent a serious and common condition. Current therapies are based on mechanical treatment. With increased knowledge of the biochemical mechanisms responsible for aneurysm expansion, it may be possible to prevent the growth of small aneurysms.

Methods: A series of experiments performed in the investigator’s laboratory during the past decade is outlined to show the evolution of our concepts of the processes underlying aneurysm formation and progression.

Results: Our understanding of aortic aneurysms has changed dramatically. Once thought to represent a simple degenerative process, aneurysm tissue is highly active metabolically with ongoing synthesis and degradation of matrix proteins. Several members of a family of matrix-degrading enzymes play an important role in this process. These enzymes can be inhibited by the antibiotic doxycycline.

Conclusions: With a better understanding of aneurysm pathology, it may be possible in the future to inhibit the growth of small aortic aneurysms before they reach a size at which the risk of rupture is significant. © 2004 Excerpta Medica Inc. All rights reserved.

Keywords: Aortic aneurysm; Collagen; Doxycycline; Elastin; Matrix metalloproteinase; Metalloproteinase inhibitor

Twenty years ago, elective treatment of peptic ulcer disease was a common surgical problem. Volumes have been written about a variety of surgical procedures for ulcers, each with its own purported benefits and problems. Recognition of the two types of histamine receptors occurred followed by selective inhibition of the H2 receptor in 1972 [1,2]. With Food and Drug Administration approval in 1977 and eventual widespread use of the first selective histamine blocker, cimetidine, the treatment of peptic ulcer disease was forever changed. Medical therapy, previously expected to prevent recurrent ulcers in a minority of patients, now became effective in most every case. The surgeon’s role was relegated to the rare patient with refractory disease or an emergent operation in an untreated patient presenting with bleeding or perforation. This progression from surgical to medical therapy was made in part because of the contributions of surgeons and scientists toward understanding the pathophysiology of peptic ulcer disease.

During the past 2 decades, surgeons and scientists have studied and learned a great deal about the pathophysiology of aneurysmal disease of the aorta. As we have begun to understand this process, opportunities for medical intervention to prevent the growth of small aneurysms may be on the horizon. Could the evolution of the treatment of peptic ulcer disease represent a paradigm for future treatment of aneurysmal disease? In my talk today, I’m going to focus on the evolution of our thinking about aneurysms. I’ll do this from the perspective of our own research done during the past 14 years because I know this work best. This is not meant to ignore seminal work by other researchers including David Tilson, William Pearce, Peter Libby, Janet Powell, Robert Thompson, and others who have moved the field forward.

Among the most important fundamental discoveries made in aneurysm research was the realization that aortic dilatation was not simply the final stage of a degenerative process. For many years, the process of aneurysm formation was considered akin to the process of a bulge forming on a worn tire. Were this an accurate portrayal, the wall of a 4- to 5-cm aortic aneurysm would be paper thin, and rupture would occur long before an aneurysm reached 5.5 cm, the current threshold for intervention. How then can the aorta increase in diameter from 2.5 to 5 cm with almost no risk of...
showed a dramatic increase in matrix proteins [3] (Fig. 1). 2 groups with atherosclerosis, but the aneurysm group expected to be similar in all 3 groups. This was true of the content of elastin and collagen, the major structural proteins in the aortic wall would be of expansion (the worn tire analogy), the total amount of the aorta. Were aneurysm formation truly a simple process, we may have the opportunity to intervene pharmacologically in a way that promotes stability and prevents progressive growth of a small aneurysm.

What is the evidence that early aneurysm expansion involves synthesis of matrix proteins? In collaboration with Bruce McMannus, a well-known cardiovascular pathologist, we had the opportunity to compare autopsy specimens of infrarenal aorta. Using a large number of specimens collected by McMannus, we looked at elderly patients with minimal atherosclerosis of the infrarenal aorta, elderly patients with severe atherosclerosis but no aneurysmal dilatation, and patients with aortic aneurysms. We measured the content of elastin and collagen, the major structural proteins of the aorta. Were aneurysm formation truly a simple process of expansion (the worn tire analogy), the total amount of these structural proteins in the aortic wall would be expected to be similar in all 3 groups. This was true of the 2 groups with atherosclerosis, but the aneurysm group showed a dramatic increase in matrix proteins [3] (Fig. 1). This was mostly caused by increased collagen, but there was also a small increase in total elastin content.

The aorta is made up of lamellae of elastin and collagen in the media surrounded by a collagenous network of adventitia. This orderly structure is completely altered in aneurysm tissue. Although one might speculate that these changes are secondary to wear and tear, the structural proteins of the aorta are quite resistant to degradation and have very long half-lives. Therefore, this loss of normal integrity occurs at least partly through active degradation of aortic structural proteins. A limited number of enzymes can degrade fibrillar collagens (types I, III, and V primarily) and elastin. Enzymes that can do this come from three different families including serine proteases, matrix metalloproteinases (MMPs), and cysteine proteases. The serine protease—called neutrophil elastase—efficiently degrades elastin, but a little of this enzyme is present in aneurysm tissue, at least in the later stages of the disease when specimens might be collected during operative repair. Both cysteine and matrix MMPs are present in increased amounts in aneurysm tissue.

How do we know which of these proteases is responsible for aneurysm formation? We focused our past work on the MMPs. From among a family of 20 or so enzymes, we narrowed our scope to 2 of the most abundant elastin-degrading MMPs: MMP-2 and MMP-9. We began this study by collecting fresh infrarenal aortic tissue from minimally diseased controls (rapid autopsy collection or transplant donors); from patients undergoing surgery for aortic occlusive disease (AOD; obstruction of the aorta from atherosclerosis without aneurysmal dilatation); and from patients undergoing open aneurysm repair (abdominal aortic aneurysm or AAA). We isolated both the protein and RNA and began the analysis using a technique called competitive polymerase chain reaction to compare the messenger RNA levels of each of these proteins. Contrary to what other investigators have reported, we found only a small increase in MMP-9 when we compared AOD with AAA. We found MMP-2 mRNA to be increased [4] (Fig. 2). We then looked at the protein levels. We initially did not find corresponding differences in the MMP-2 protein levels until we learned from our collaborator, Hideaki Nagase, that MMP-2, when activated, can be tightly bound to matrix. After using more stringent extraction techniques that allowed for extraction of this matrix-bound fraction, we saw the same increases in

Fig. 1. Increased metabolic activity in AAA tissue. Protein content was determined in a 1-cm ring of aortic tissue taken from autopsy specimens with minimal disease (controls), from specimens with AOD but no aneurysm, and from specimens with AAA (n = 8/group). AAA = abdominal aortic aneurysm; AOD = aortic occlusive disease. *Greater than control, P < 0.05. #Greater than AOD, P < 0.05.

Fig. 2. MMP-2 mRNA levels in aortic tissues. Total RNA was extracted from specimens with minimal disease (controls), from specimens with AOD but no aneurysm, and from specimens with AAA (n = 5/group). Control, AOD, and AAA mRNA were analyzed by quantitative competitive reverse transcriptase–polymerase chain reaction. The mean ± SEM MMP-2 mRNA levels (pg/μg total RNA) from each group are shown in the bar graphs. AAA = abdominal aortic aneurysm; AOD = aortic occlusive disease; MMP = matrix metalloproteinases. *Different from control (P < 0.05). #Different from AOD (P < 0.05).
MMP-2 protein from AAA tissue that had been seen at the RNA level. Importantly, we discovered that much of this bound protein had been in the smaller, processed active form (Fig. 3). We concluded from this work that MMP-2 might play a role in aneurysm formation.

Admittedly it is tempting to take this kind of information and overstate the conclusion that MMP-2 causes aortic aneurysms. These data showed an association between AAA and MMP-2 but no cause-and-effect relationship. Keep in mind that by the time we harvest aneurysm tissue at surgery, many changes have already occurred in the matrix structure (remodeling) and cellularity (decreased number of resident mesenchymal cells and increase number of inflammatory cells) of the aorta. Could the increase seen in MMP-2 (or other enzymes identified by other groups) be secondary to the process, representing an epiphenomenon, rather than having a role in the etiology and progression of aneurysms? The answer is yes it could. How could we then determine what factors might have an important role in aneurysm formation? We realized at this point that we needed an animal model to answer this question.

As we investigated the aneurysm models that had been developed in the past, we focused specifically on the histologic changes that occurred in these models. We particularly liked a model first described by Gertz et al [5] in the rabbit carotid artery. These investigators found that by applying calcium chloride to the outside of an artery, an inflammatory process ensued. These inflammatory cells were a mixture of lymphocytes and macrophages, which are precisely the same types of inflammatory cells found in human aneurysm tissue. Subsequent to the inflammation, the artery enlarged. We chose this model but wanted to move from the rabbit carotid artery to the mouse infrarenal aorta. Jason Rehm and Matt Longo, surgery residents working my laboratory, spent more than a year addressing each of the following hurdles in a stepwise fashion: (1) exposing and isolating the aorta without causing exsanguination; (2) determining the concentration of calcium chloride and the duration of exposure; (3) determining the time for aneurysm development after injury; and (4) determining the temporal changes in cell type and expression of MMPs [6]. We were pleased to find out, after all this work, that this model recapitulated the important features of human AAA tissue including macrophage and lymphocyte infiltration, increased MMP-2 and MMP-9 expression, and matrix destruction.

Once this model had been characterized, we went back to the question of whether MMP-2 or MMP-9 was responsible for the formation of aneurysms. Although developing a mouse model of aneurysms was a technical challenge, we chose to do this to take advantage of genetically engineered mice in which expression of one single gene has been deleted. These are called “knockout mice” because expression of the targeted gene had been deleted or knocked out. Although deletion of some genes can cause important developmental anomalies, the MMP-2 and MMP-9 knockout mice are phenotypically normal with normal aortic histology. When we induced aneurysms in these mice, no aneurysms formed in the MMP-9 knockout mice, confirmed an earlier finding of Thompson’s group using a very different mouse model [7] (Fig. 4). The MMP-2 knockout mice were also completely resistant to aneurysm formation. Both enzymes, MMP-2 and MMP-9, play a critical role in aneurysm formation, and because both are completely effective, they appear to somehow work in sequence. Understanding pre-
cisely how these enzymes interact in this process will re-
quire more work, and these data show that other enzymes
may play a critical role in the pathway to AAAs. Because
MMP-9 is produced by macrophages, we wondered if we
could reconstitute an aneurysm in the MMP-9 knockout
mouse by infusing competent macrophages from the wild-
type mouse. Indeed, when the normal macrophages were
given intravenously before and after the calcium chloride
injury, the MMP-9 knockout mouse developed an aneurysm
(Fig. 5). This was not the case when we gave competent
macrophages to the MMP-2 knockout mouse, probably be-
cause MMP-2 is mainly a product of smooth muscle cells
and fibroblasts.

Although these data are important in terms of under-
standing factors that have a role in aneurysm development,
we don’t yet have the opportunity to efficiently and safely
“knockout” MMP-2 or MMP-9. Other options exist, al-
though they offer less specificity. There is a family of
hydroxamate-based MMP inhibitors that are quite potent.
There was great enthusiasm for these drugs as they were
tested in cancer trials under the working hypothesis that
cancer cells require MMPs for invasion of surrounding
tissues. However, side effects were relatively common and,
importantly, the hydroxamate-based MMP inhibitors were
not particularly effective in treating cancer.

The tetracyclines are a family of antibiotics that were
widely used in the 1970s. In 1983, Golub [8], a dental
researcher, made an important observation. He found that
the tetracyclines could inhibit MMPs, and his subsequent
work has shown that this was unrelated to the antibiotic
that doxycycline inhibited aneurysm formation in a rodent
model of aneurysms. We subsequently confirmed this im-
portant observation in our mouse model (Fig. 6). We also
showed that aneurysm inhibition could be achieved at serum
doxycycline levels that were measured in patients taking
standard doses of doxycycline (200 mg/day) [10]. In col-
laboration with a group of investigators interested in the
medical treatment of aortic aneurysms, we conducted a
safety trial showing that doxycycline was well tolerated in
AAA patients [11]. A small randomized trial from Scandi-
navia has suggested that doxycycline can inhibit expansion
of small AAAs.

As I stated at the beginning of this talk, a number of
surgeon and scientists, using both clinical and basic science
techniques, have added important pieces to this puzzle of
AAAs. This work has led to a point where we will be able
to begin testing medical therapy in large randomized trials.
AAAs are easily and inexpensively detected by ultrasound.
In fact, such screening programs have detected aneurysms
in 3% to 9% of patients screened. Screening, however, is not
considered cost effective because 9 out of 10 of aneurysms
detected are below the threshold that requires immediate
intervention. We have no therapy to offer the 9 patients with
small aneurysms, and they are left with the anxiety of
knowing that they have a progressive and potentially fatal
disorder. Early detection and effective medical therapy
would completely change the current approach to AAAs.

References

[3] Minion DJ, Davis VA, Nejezchleb PA, et al. Elastin is increased in


